MATURATION, NATURAL DEATH AND THE PROLONGATION OF THE LIFE OF UNFERTILIZED STARFISH EGGS (ASTERIAS FORBESII) AND THEIR SIGNIFICANCE FOR THE THEORY OF FERTILIZATION.

JACQUES LOEB,

HULL PHYSIOLOGICAL LABORATORY, UNIVERSITY OF CHICAGO.

I. Introduction.

I have pointed out in my earlier publications that fertilization of the egg serves to prolong the life of the egg.[1]

The mature unfertilized egg dies in a comparatively short time. Because of this fact the egg becomes of importance as an object of experiment, to study the question of natural death and prolongation of life. For by no means has it been decided that there is a "natural" death. We only know that with an increase in age a critical period is reached in which every living organism dies under the influence of conditions which do not affect a younger organism. It may, therefore, be of interest that we are able to show, as I believe, that a critical period exists in the life of many eggs in which they die a "natural" death, and that the life of the eggs can, during this period, be saved or lengthened only through various external conditions.

The egg of the starfish (*Asterias Forbesii*) serves as a very favorable object of experiment in the study of this question. When removed from the ovary this egg is generally "immature," but as soon as it comes in contact with sea water it begins to "maturate."

Morphologically, the immature state is characterized by a very large, plainly visible nucleus.[2] The process of maturation consists

[1] Loeb, J., *American Journal of Physiology*, Vol. IV., 1901; Loeb and Lewis, *ibid.*, Vol. VI., 1902.

[2] The recent beautiful experiments of Delage have shown that, beside these visible changes in the nucleus, chemical, but morphologically invisible, changes also occur in the protoplasm. Delage, Études expérimentales sur la maturation cytoplasmique et sur la parthénogenèse artificielle chez les Échinodermes, *Arch. de Zoologie expériment.*, Vol. IX., 1901.

morphologically in this, that the nucleus becomes invisible and the polar bodies are thrown out.

This process is completed within one or two hours after the eggs are removed from the ovaries and placed in sea water. Only when maturation is complete is it possible to cause the egg to develop through the addition of sperm or through the physical and chemical agencies that have been described by me, Delage, Mathews, and Greeley.

II. The Natural Death of the Mature Unfertilized Starfish Eggs.

The living eggs of *Asterias* are light yellow in color and homogeneous. They retain this appearance during the process of maturation as long as they are alive. They retain this appearance also when they are made to develop through the entrance of a spermatazoön or through the proper chemical or physical means.

If, however, the mature eggs are not fertilized or do not develop, they die in the course of four to twelve hours, and this process of dying is accompanied by a characteristic change in the color of the egg. The egg becomes at first opaque, then almost black, the homogeneous structure of the protoplasm becomes granular. If such a culture of unfertilized eggs is examined under the microscope after twenty-four hours, two kinds of eggs are found, first, the just described dark, dead eggs *which are mature,* and secondly, *living* normally colored *but immature* eggs. For usually not all the eggs that are removed from the ovaries of a starfish maturate at once; many maturate very late, others not at all. It is readily seen *that the immature eggs remain alive for several days until they finally become the prey of bacteria; while the mature eggs become opaque and die in four to twelve hours after maturation has been completed.*

Is the death of the mature but undeveloped egg brought about through internal conditions or through the bacteria contained in the sea water?

A trustworthy way of determining this consists in making sterile culture of the eggs in sea water. This is a relatively simple procedure in the case of starfish. Eight flasks were sterilized, filled with sterilized sea water, and again heated for twenty

minutes on three successive days to 100°C. A female starfish was thoroughly washed externally, an arm was opened, and one of the ovaries removed with sterilized forceps and placed in sterilized sea water. From the thick stream of eggs which at once flowed out of the ovary, a few drops were quickly introduced with a sterilized pipette into each of the sterilized flasks. A second series of eight flasks contained normal sea water, and a few drops of the same eggs were introduced into these flasks also. A third series of flasks were filled with sea water, to each of which were added 2 c.c. of a putrid, foul-smelling culture of old starfish eggs in order to bring about a rapid development of bacteria from the beginning. Each of these flasks also contained eggs from the same culture as those in the sterilized flasks.

That perfect sterilization had been attained in the first eight flasks was proved by the fact that all the flasks remained absolutely clear and cloudless during the course of the experiment, and that three of the flasks which had not been opened are even to-day (after six weeks) absolutely clear, and every egg can be individually recognized. The flasks containing the unsterilized sea water became cloudy already after twenty-four hours, and after two days the eggs had become the prey of bacteria and no individual egg could be recognized. The sterilized flasks which were opened were at all times free from foul odor, while the unsterilized flasks gave off a penetrating stench, often after one, invariably after two days. The microscopic examination of the sea water for bacteria was always negative in the sterilized flasks, always positive in the other flasks. In those flasks to which 2 c.c. of the putrid culture of starfish eggs had been added, bacteria and infusoria were exceedingly numerous from the beginning.

Six hours after the beginning of the experiment one flask of each of the three series was opened and the eggs examined microscopically. The picture was the same in all three flasks. Nearly all the eggs were mature, and a small number of them were opaque or black. But what is of the greatest importance to us is the fact that the percentage of opaque dead eggs was just as great in the sterile culture (if not greater) than in the unsterilized or the infected sea water.

Twelve hours later, that is to say eighteen hours after the

beginning of the experiment, one of the flasks of each of the three cultures was again opened. At this time nearly all the eggs of the sterile culture were opaque or black, and a few were already granular. In the two other cultures an equal percentage of the eggs were opaque. The eggs, therefore, die just as rapidly in the sterilized flasks which are absolutely free from bacteria as in the flasks containing bacteria. Death follows from internal causes, and so rapidly that the few bacteria in the sea water are scarcely able to accelerate the death of the eggs. The eggs have already died from internal causes before the bacteria can attack them in sufficient numbers to threaten their existence.

The flasks which were opened later served only to corroborate what has been said. The experiment was repeated with the same result. Each of the flasks that were opened during the first few days also contained a small number of living transparent eggs. *The latter were, without exception, immature. The experiment, therefore, shows that the mature eggs of starfish die in the course of a few hours, and that the cause of this death cannot be sought in the bacteria of the sea water; and further, that under exactly the same conditions the immature eggs remain alive.*

III. The Chemical Conditions Necessary for Maturation in Starfish Eggs.

Since the eggs of *Asterias* are usually immature in the ovary, but, in part, at least, maturate in the course of one or two hours when introduced into sea water, the suspicion was aroused that some of the substances contained in the sea water brought about the maturation. In order to determine which substance this might be, a series of solutions were prepared having approximately the osmotic pressure of the sea water. The result was so simple that it is not necessary to describe all the experiments here. For it was found that when the eggs are introduced into solutions which contain free hydroxyl ions, maturation soon follows, but that this does not occur in solutions containing no hydroxyl ions. So, for example, the eggs retain their nucleus in a $5/8 n$ NaCl solution, or in NaCl solutions to which some potassium or calcium has been added. If, however, .5 to 2 c.c. $n/10$ NaOH is added to each 100

c.c. of such solutions, maturation soon follows; that is to say, the nucleus becomes invisible. Since sea water contains free hydroxyl ions the conclusion is justified that these are one of the causes for the maturation of the starfish egg. It was possible to prove this assumption through further experiments. If a small amount of acid is added to sea water, the free OH ions disappear, and the water becomes acid in reaction (through the addition of 1.5 c.c. or more $n/10$ HCl to 100 c.c. sea water). Immature eggs were introduced directly into sea water to which 1, 2, 3 and 4 c.c. of an $n/10$ HNO_3 solution had been added to each 100 c.c. of sea water. While, as is usual, a large percentage of eggs soon maturated in the normal sea water, maturation did not occur at all in the vast majority or in all the eggs contained in the sea water to which 2 or more c.c. acid had been added. The addition of even 1 c.c. of acid diminishes the number of eggs that maturate. But it is not even necessary to keep the eggs permanently in neutral or acid sea water in order to inhibit maturation. If 4 or 5 c.c. of a $n/10$ HNO_3 solution are added to 100 c.c. sea water, and immature eggs are introduced into such a solution for only about fifteen minutes, relatively few eggs maturate when they are returned to normal sea water. Such acidified sea water does not kill the starfish eggs.

We shall see later that the procedure described here which, when used upon immature eggs, prevents maturation, brings about artificial parthenogenesis when used on mature eggs.[1]

I have, moreover, been able to convince myself of the fact that the eggs which are introduced into acidified sea water in an immature state, can be fertilized by sperm if they finally maturate. It is possibly in harmony with what has just been said that the addition of $NaHCO_3$, or larger amounts of sodium citrate to the sea water accelerates the process of maturation. Free hydroxyl ions are present in the solutions of both substances, and it is possible that their addition to the sea water increases the concentration of the free hydroxyl ions in the sea water.

But the hydroxyl ions are certainly not the only substances in the sea water which favor or cause the maturation of the starfish egg. I soon found that when different specimens of eggs are

[1] Loeb, Fischer, and Neilson, *Pflüger's Archiv*, 1901, Bd. 87.

taken from the same culture, and the percentage of mature eggs is determined, this percentage is subject to the greatest variations. The cause of these variations was soon discovered. For it was found that where the eggs lie together in a heap maturation occurs slowly, but where they lie in a thin layer, maturation occurs quickly. This fact suggested the importance of oxygen for maturation. Where the eggs lie in a heap the appropriation of the oxygen by the superficial layers of eggs prevents the diffusion of the oxygen to those lying deeper.

Experiments were now made in which the oxygen of a small flask containing a small amount of sea water was replaced by hydrogen. When, in such experiments, all the oxygen was entirely removed maturation did not occur in any, or at least the majority of the eggs, in spite of the presence of the hydroxyl ions in the sea water. There are, therefore, at least two substances in sea water which cause or accelerate maturation, oxygen and hydroxyl ions. Possibly other constituents of the sea water are also concerned in the process, but NaCl, Ca, and K have apparently no beneficial effect upon maturation.[1]

It seems, therefore, that the absence of oxygen and hydroxyl ions in the ovaries belongs to the conditions which inhibit maturation of the eggs in the ovary.

IV. The Prolongation of the Life of the Unfertilized Starfish Egg by the Prevention of Maturation.

We have shown above that the mature eggs of a culture of unfertilized starfish eggs die within a short time (which decreases with an increase in temperature), while the immature eggs remain alive a relatively long time. It was necessary now to show that when the maturation of a culture of unfertilized egg of *Asterias* is prevented artificially, the eggs live longer. We begin with the experiment which is technically most simple. The eggs streaming from the ovary are divided into two portions. One portion of eggs is carefully distributed without mechanical agitation, by carefully tipping the vessel, in a thin layer over the bottom

[1] Professor Whitman informs me that the maturation of the eggs of *Clepsine* does not begin until after they are laid. Possibly the oxygen contained in the water is in this case also a necessary condition for maturation.

of the vessel. The vessel must be low and the layer of sea water covering the eggs not too deep, so that the diffusion of oxygen to the eggs can occur with ease. A second portion is introduced with just as great care into a small-calibered glass tube sealed at one end. This glass tube is half filled with eggs, so that one is certain that the lower layers of the eggs in the pipette receive little or no oxygen. It is self-evident that the eggs must be introduced into the tube immediately after being laid. When, after twenty-four hours, the eggs which are distributed over the bottom of the glass dish and which receive a large amount of oxygen are compared with those at the bottom of the glass tube, a striking difference is found between them. The eggs richly supplied with oxygen contain a much larger percentage of mature dead and black eggs than those kept in the lack of oxygen. In the latter the living immature eggs are in the majority, and a part of these maturate when spread out in a thin layer over the bottom of a vessel. These experiments are also well adapted to show that the rapid death of the mature unfertilized sea-urchin eggs is determined through internal conditions and not by the bacteria contained in the sea water. I will cite an example.

One portion of a lot of eggs was spread out in a thin layer over the bottom of a dish; another was heaped in a mass in the same dish. The sea water was the same in both cases. The first portion of eggs maturated in a few hours and were, in less than twelve hours, opaque and dead, while the water was still absolutely clear and without odor of putrefaction. After twenty-four hours the water became putrid and contained many bacteria. Even after three days, when the water was exceedingly foul and cloudy, a portion of the eggs which had lain in a heap, that is to say, without oxygen, *were immature and living*. They were introduced into fresh water and spread out into a thin layer. They maturated and developed into swimming larvæ upon the addition of sperm. It is self evident of course that even immature eggs finally become the prey of bacteria, and so go to pieces in the sea water.

The same experiment can be made in a somewhat more complicated way with pure oxygen and hydrogen. The freshly laid eggs

of a starfish were distributed into two series of eight flasks. The one series of flasks was connected with a hydrogen generator; the other with a tank containing pure oxygen. Before the beginning of the experiment all the air in one of the series of flasks was driven out by the current of hydrogen. During the course of the experiment a vigorous current of hydrogen was maintained. Both series of flasks contained freshly laid immature eggs of *Asterias*. The experiment lasted three days, and from time to time a flask was removed and its contents examined. The eggs which had been exposed to the current of oxygen maturated just as rapidly and as numerously as those in ordinary sea water, and the mature eggs soon died. In the current of hydrogen maturation did not occur in the majority of the eggs, and these remained alive. In the hydrogen cultures a rapid development of bacteria occurred while in the oxygen cultures this occurred to a small degree.[1]

Treatment with acids which, as we have shown above, prevents the maturation of the eggs (without killing them) also prevents their death and disintegration.

Eggs which, without having been in contact with pure sea water, are introduced for ten or fifteen minutes into 100 c.c. sea water plus 4 c.c. $n/10$ HCl maturate very slowly or not at all when they are returned to normal sea water. They also retain, as long as they are immature, the transparent, normal appearance of living eggs until they finally become the prey of bacteria.

It seems to follow from these experiments that the same processes which underlie the maturation of starfish eggs also lead to their death (if they are not inhibited through circumstances which we designate by the term fertilization). I tried to see, now, whether it was also possible to maintain the life of the *mature* egg through lack of oxygen. I indeed obtained in a few cases positive results in this direction. The eggs of a starfish were spread in a thin layer over the bottom of a dish. After three hours seventy-five per cent. of the eggs were maturated. A portion of the mature eggs were carefully introduced into the

[1] Care must be taken in these experiments that the air is thoroughly removed from the sea water in the hydrogen flasks before the eggs are introduced into them. Of course the hydrogen apparatus must also be free from air.

glass tube described above, in which the deeper layers suffered from lack of oxygen. A second portion was introduced into a small flask through which a steady stream of pure oxygen was passed. On the following morning, that is to say, fifteen hours after the eggs were brought into the atmosphere of pure oxygen, the various portions of the eggs were examined. The eggs introduced into the current of oxygen showed in one vessel *98 per cent. mature and dark, dead eggs and 2 per cent. immature living* eggs. The eggs which had remained in normal sea water contained, as before, about 75 per cent. mature eggs, all of which, however, were black and dead, with the exception of a few eggs which had begun to divide,[1] and were living.

The immature eggs were also still living. Upon the other hand, the eggs which had been left in the glass tube in absolute or relatively high lack of oxygen, were nearly all living! This observation seems to show that the same processes which lead to the maturation of the egg bring about its death if they are not inhibited at the right time. In this way the process of fertilization becomes a life-saving or life-prolonging act.

V. Do these Facts Hold for Other Forms?

The question of the relation between maturation and natural death can be studied most beautifully in the starfish egg because it is possible to obtain it in an immature condition, and because maturation follows very rapidly. With sea-urchin eggs conditions are much less favorable, since the egg maturates within the ovary, and since it is difficult to obtain immature eggs during the spawning season. I have, therefore, been unable to discover which chemical factors determine the maturation of the sea-urchin egg, and to decide whether the same circumstances cause the death of the sea-urchin egg that bring about the death of the starfish egg; and whether the life of the sea-urchin egg can be prolonged through a prevention of these circumstances. In an indirect way Lewis and I attempted to answer this question last year, when we assumed that the destructive processes which bring about the

[1] This cleavage was possibly brought about through mechanical agitation; I had repeatedly shaken the dish to facilitate the introduction of oxygen into the sea water.

death of the unfertilized egg are enzymatic (autolytic?) processes which can be inhibited through poisons such as KCN.[1]

We did in fact succeed in showing that the addition of a small amount of KCN to the unfertilized starfish eggs markedly lengthens their life. Even after seven days such eggs can be fertilized as soon as they are returned to normal sea water. We also pointed out that, because of the well-known bactericidal properties of potassium cyanide, the experiments on sea-urchin eggs were not in themselves decisive and so began experiments on starfish eggs[2] which, however, we were not able to complete at that time. In dealing with eggs which are as long lived as sea-urchin eggs a great development of bacteria in normal sea water can not be prevented, since a few of the eggs always die and so serve as an excellent culture medium for the further development of bacteria. It need, therefore, surprise no one that the unfertilized eggs of sea-urchins, as I was able to show this year, live in sterile sea water for five days, or possibly longer, while they die much earlier in ordinary sea water (about two days). The very fact that the eggs of sea-urchins are found mature in the ovary indicates that they are able to live a considerable time after maturation and that they differ in this respect from the starfish egg.

It is, however, a fact that in the same sea water the fertilized and developing sea-urchin eggs live longer than the unfertilized eggs.

It almost seems as if in certain of the higher animals there are eggs which develop only when they are fertilized immediately after leaving the ovary. Under the direction of Professor C. O. Whitman, Harper has shown that the eggs of pigeons are fertilized the moment they leave the ovary. The sperm lives in a gelatinous mass upon the surface of the ovaries,[3] so that provision is made for the necessary contact between sperm and egg. This also does away with the difficulty which many have found in

[1] Loeb and Lewis, *American Journal of Physiology*, Vol. VI., 1902.

[2] Loeb and Lewis, *American Journal of Physiology*, Vol. VI., 1902.

[3] Spermatozoa are in general much longer lived than eggs, even though great differences exist in this regard in different animals. In the spermatic vesicles of the queen bee spermatozoa are believed to remain more than a year after copulations.

explaining how the spermatazoön finds its way to the egg in animals in which fertilization occurs within the body. Definite directive forces are clearly not necessary, since a portion of the spermatazoa must reach the ovary, through their ciliary motion, by way of the uterus and Fallopian tubes. Experiments similar to those made by Harper upon pigeons must yet be made upon mammals. Yet there seems to be no doubt that the mammalian egg of many species is also fertilized before it reaches the uterus. Cases of extra-uterine pregnancy also point to the possibility that fertilization may occur at the surface of the ovary.

VI. The Prolongation of Life and the Theory of Fertilization.

Our experiments seem to have proved that the mature unfertilized starfish egg dies within a few hours through internal changes but that the process of fertilization saves the life of the egg. This is true, not only of the fertilization of the starfish egg by spermatozoa, but also for the chemical fertilization through hydrogen ions. Mr. Neilson succeeded this year in keeping the parthenogenetic larvæ of starfish alive much longer than has thus far been the case (over thirty days), and Dr. Fischer was able to accomplish the same for the larvæ produced osmotically from unfertilized sea-urchin eggs. It is therefore possible that the chemical or osmotic fertilization of these eggs can give rise to as long-lived larvæ as the fertilization of the egg through sperm.

But how does the spermatozoön or the physical and chemical means substituted for it, save the life of the egg and why does the mature egg die when it is not fertilized by sperm or artificial means? I believe that the answer lies in this, that the fertilizing agencies accelerate metabolic processes in the egg which, before fertilization, went on only slowly. After fertilization by sperm or by the chemical or physical means substituted for it, the egg divides and grows, which it did not do before fertilization occurred. Growth is inconceivable without a preponderance of synthetical over hydrolytical processes. I believe it possible that the determining factor in the chemical forces set in motion within the egg through fertilization consists in this that the synthetical processes in the egg are accelerated. If these processes are not inaugurated

or accelerated the egg dies. The wasting of the body in old age also indicates a decrease in synthetical processes. Whether the second critical period occurring in old age is similar to the critical period of the egg cannot yet be determined. Yet it is not impossible that the question of the prolongation of life at this period should pass over into the question of the possibility of accelerating synthetical processes.

We, therefore, come to the conclusion that fertilization accelerates a series of chemical changes (syntheses?) in the egg which do not occur sufficiently rapidly without spermatic, chemical or osmotic fertilization in the eggs of the majority of animals. But why does the mature egg die when these processes are not accelerated, and why does it remain alive before it maturates? The egg must often exist for years in the immature condition in the ovary. In answer I can only suggest *that the processes underlying maturation are at least in some form of a destructive nature (one might think of autolytic processes) which the egg cannot withstand for an indefinite length of time without dying.* In many eggs the velocity of these destructive (autolytic?) processes may be greater than in others and this may determine the differences in the velocity with which the mature, unfertilized egg dies. It is in harmony with this view that when maturation is prevented, or the mature egg is put under condition which inhibit the process of maturation or the chemical processes underlying it, that the life of the egg is lengthened. Lack of oxygen or the addition of an acid work in this way in the case of starfish eggs; a slight addition of potassium cyanide in the case of starfish and sea-urchin eggs. But since all of these substances injure the eggs indirectly and do not entirely do away with the destructive (autolytic?) processes occurring within the egg, life is not prolonged to the same extent by these means as by fertilization in which case life is prolonged not only through an inhibition of the destructive but also through an acceleration of the synthetical processes.

That the chemical processes which underlie maturation are not identical with those which bring about fertilization seems to be supported by the observation made above, that the same means —the treatment with acid—which causes the mature egg to

develop and live beyond the bipinnarian stage, inhibits the maturation of the immature egg. When the *mature* unfertilized eggs of a starfish are introduced for fifteen to sixty minutes into a mixture of 100 c.c. sea water plus 3 c.c. $n/10$ HCl ninety per cent of the eggs can, under favorable conditions, develop into larvæ. If, however, the eggs are introduced into such a solution for the same length of time *before maturation,* the maturation of the eggs is prevented either permanently or for a long time.[1] The difference is still more striking when the eggs are kept for a shorter time in a mixture of 100 c.c. seawater and 5 c.c. $n/10$ HCl. This shows that acid affects the process of development and the process of maturation in opposite or at least different ways.

We must now raise the question, How does the behavior of naturally parthenogenetic eggs, such as the eggs of bees harmonize with these ideas?

In naturally parthenogenetic eggs it seems as if the processes which underlie maturation pass over into those underlying development. But it is possible that this is only apparently the case, and that in reality it so happens that in the processes underlying maturation a metabolic product is formed in the parthenogenetic animals which favors the processes of development. We know that an exceedingly small amount of hydrogen ions suffices to bring about development in unfertilized starfish eggs; that an exceedingly small amount of calcium causes the unfertilized eggs of *Amphitrite* to develop; and that a trace of potassium ions brings about the development of unfertilized *Chætopterus* eggs.[2] It is entirely possible that the specific ions or other substances necessary to start the development of the eggs of the bee are formed within the egg itself through the chemical changes taking place during or after maturation, and that without the formation of these substances development is impossible. In the case of sea-urchins and starfish eggs one might also believe that the processes of maturation and the processes of development pass over into each other. For it has often been observed that the

[1] The eggs which finally maturate in spite of the previous treatment with acid often begin to cleave when maturation is complete and develop into larvæ, while the control eggs kept in normal sea water do not develop.

[2] Loeb, Fischer and Neilson, *l. c.*

unfertilized eggs of these forms after having resided in "normal" sea water for about twenty-four hours begin to cleave shortly before death. This cleavage, however, never goes beyond the two- or four-celled stage. This might be explained by the fact that the eggs begin to die at this time. After I had found this year that the eggs of sea-urchins can still be fertilized after a residence of five days in sterilized sea water (at summer temperature), I decided to study this question of spontaneous cleavage somewhat more closely. If it were true that individual sea-urchin eggs begin to cleave in ordinary sea water after about twenty hours, and cease to develop any further only because they soon die, it would be expected that many or all should cleave when kept alive four or five days, and that a number of them should reach a fairly advanced stage of development. A lot of sea-urchin eggs were distributed into a series of flasks containing sterile sea water. One of the flasks was opened every morning and a careful search was made for developed eggs.

In the course of five days I never found a single divided egg, either in the two-celled stage or in later stages of development. It is possible that during the last days of the experiment a few eggs divided, and that the cleavage cells fell apart. Lewis and I found last year that when eggs are fertilized forty-eight or more hours after their removal from the ovaries they form no membrane and the cleavage cells fall apart. I have corroborated this fact this year. Usually more than one embryo develops from such an egg, because the cells drop apart. I kept this fact in mind and will not disavow that a few small eggs were present, which perhaps represented only half the mass of an ordinary egg. But nearly all the eggs were of normal size, and since small eggs are occasionally found even under normal conditions, the experiment shows that in sea-urchin eggs also the processes of maturation are not continuous with those of cleavage, and that entirely different conditions which we can bring about through the abstraction of water or the entrance of a spermatozoön are necessary that division may occur. It cannot be urged that the sterilized water perhaps prevented the cleavage. When at the conclusion of the experiment these same eggs were fertilized in sterilized water by adding a drop of sperm, they developed to the pluteus stage in

sterile sea water. I, therefore, consider it possible that where authors describe a cleavage of the unfertilized sea-urchin eggs in "normal" sea water, that the sea water or the eggs had in reality suffered some change which had escaped the notice of the observers. One might think of evaporation and increase in the osmotic pressure of the sea water. A very slight increase in the osmotic pressure of the sea water is sufficient to cause the sea-urchin egg to divide into two cells in the course of twenty hours. One might also think of a change in the sea water brought about by the putrefaction of the dead eggs. Finally it is possible that a substance is perhaps formed (for example, an acid) in the dying eggs which brings about a single cleavage.

The relations which exist between maturation and natural death upon the one hand, and fertilization and the prolongation of life upon the other, lead us to the conclusion that a "fertilization" must perhaps come to pass in every egg, even in those naturally parthenogenetic. Only according to our idea, the act of fertilization is not identical with the morphological process which is designated fertilization. It is rather a chemical or a physico-chemical act which accelerates certain (synthetical?) metabolic changes in the egg, which occur in the egg under ordinary conditions also, only much too slowly. The difference between naturally parthenogenetic eggs and the eggs which must be fertilized before they can develop consists perhaps in this, that to the latter the catalytically working substance or complexus of conditions must be added from the outside in order to accelerate the synthetical (?) processes, while in the naturally parthenogenetic eggs these substances are formed within the eggs (possibly in conjunction with the processes of maturation).

The connection between the prolongation of life and fertilization clearly points out that every purely morphological theory of fertilization is incomplete and that a correct theory of this process must have a physco-chemical basis. The means of reaching this basis I see in further attempts at causing development of unfertilized eggs through unequivocal physical and chemical means.

VII. Conclusions.

1. Our observations and experiments seem to show that in the same sea water and under otherwise identical conditions, *mature but unfertilized* starfish eggs soon die, while immature as well as mature but *fertilized* eggs live longer.

2. It seems certain that the rapid death of the mature unfertilized starfish eggs is determined by internal conditions connected with maturation and not by the bacteria contained in the sea water. The proofs for this are: First, mature eggs die just as rapidly in sterilized sea water free from bacteria as in unsterilized water, and secondly, when maturation is prevented artificially the eggs may continue to live in water containing many bacteria.

3. We have shown that oxygen and free hydroxyl ions accelerate the maturation of starfish eggs; that lack of oxygen and a neutral or faintly acid reaction of the sea water inhibit or prevent maturation. The fact that the eggs which remain immature in the ovaries of the starfish maturate when brought into sea water seems to find its explanation in part at least through this.

4. When the maturation of starfish eggs is prevented artificially through lack of oxygen or the addition of an acid to the sea water they remain alive much longer than when they maturate. The eggs in which maturation has already begun or has just been completed seem also to be saved from rapid death by these means.

5. It seems to follow from these facts that the same chemical processes do not necessarily underlie the process of maturation and the process of fertilization. Fertilization by spermatozoa, chemical or physical agencies lengthens the life of the egg, while the changes following the maturation of the egg lead, sooner or later, to death (through autolysis?). It is in harmony with what has been said that the same treatment with acid which brings about artificial parthenogenesis in mature starfish eggs inhibits the process of maturation when used upon immature starfish eggs.

7. These facts corroborate a suggestion which I have made before, that the fertilizing action of the spermatozoön consists in this, that it carries into the eggs substances which accelerate the course of certain (synthetical?) processes in the egg. Such an acceleration might, for example, be brought about through certain ions (for example, the hydrogen ions of nucleic acid), yet the

possibility that such catalytic effects might also be brought about through enzymes or other substances is of course not shut out. Yet this fact must be considered: that we have been able to produce artificially *normal* embryos *capable of development* through ions, while the careful experiments of Gies conducted in my laboratory in which he attempted to find the same to hold for enzymes have thus far failed.

In conclusion I wish to thank my assistant, Mr. Neilson, for the assistance which he rendered me in these experiments.